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Renal clearance and isolated kidney perfusion techniques

THOMAS MAACK

Department of Physiology, Cornell University Medical College, New York, New York, USA

Renal clearance

Renal clearance techniques are in essence measurements of input–output relationships of endogenous or exogenous substances in the kidney. When judiciously applied and interpreted, clearance techniques measure whole kidney functions and, therefore, are useful to assess the effects of systemic factors or substances on total kidney function. As such, they are still an indispensable tool in the armamentarium of renal physiologists, pharmacologists, nephrologists, and clinical investigators. In addition, earlier investigators in the decades spanning the 30s to the 60s applied renal clearance techniques to gain insights as to nephron sites and mechanisms of renal transport processes [1]. Some of the most brilliant inferences in our field come from such early application of the clearance techniques, and they still serve as inspiration for many of the research efforts of present day investigators. With the advent of more direct techniques at the tubular, cell, membrane and molecular levels, such indirect use of clearance techniques rightfully lost some of its appeal. Nevertheless, because of their simplicity and power, clearance techniques are still useful, on the one hand, to direct the application of more modern technologies and, on the other hand, to integrate the information obtained with the latter into a coherent description of whole kidney function.

In the first part of this article, I will present some of the issues regarding the definition, application, and interpretation of clearance techniques without an attempt to cover the field. For a detailed analysis of clearance techniques, the reader is referred to the excellent review articles by Levinsky and Levy [2] and by Schuster and Seldin [3], which also contain extensive bibliographical references.

The first issue to be addressed is the definition of the term "renal clearance". The term was defined by Moller, McIntosh, and Van Slyke [4], Holten and Rehberg [5], and Homer W. Smith [6], as the ratio of $U_x \cdot V\ P_x$, where U_x and P_x are the urine and plasma concentrations of a substance $\underline{x}$ and $\dot{V}$ is the urine flow rate. Conceptually, textbooks also use the two descriptive definitions by Homer W. Smith: "clearance is the volume of plasma required to supply the quantity $\underline{x}$ excreted in urine each minute time" or "clearance is the virtual volume of plasma completely cleared of that substance in one minute's time" [6]. The clearance equation and the first of the above definitions have to be understood as referring specifically to substances which are cleared from the plasma by the kidney exclusively via the urinary excretion route, and are not valid for substances which are metabolized or synthetized by the kidney [1, 2, 6]. A case in point is the clearance of low molecular wt proteins and peptide hormones. It has been demonstrated that the kidney is the major organ to remove these substances from the circulation, a function which contributes in a major way to the regulation of the plasma levels of these substances and which is accomplished essentially by renal filtration, uptake by proximal tubular cells, and hydrolysis of the proteins within these cells [7]. The renal clearance of low molecular wt proteins and peptides, as defined by $U_x \cdot \dot{V}/P_x$, is negligible while the actual plasma clearance of these substances by the kidney may be as high or higher than the inulin clearance [7]. This is true also for substances which are metabolically utilized by the kidney to significant degrees, such as glutamine, lactic acid, and intermediates of the Krebs cycle [1]. Ideally, the term renal clearance should be defined in its generalized form by the product $E_x \cdot RPF$ where E_x is the fractional renal extraction of $\underline{x}$ $(A_x - V_x/A_x)$ and RPF is the renal plasma flow. In the particular conditions in which $\underline{x}$ is extracted from plasma exclusively via the urinary excretion rate, mass conservation demands that $E_x \cdot RPF = U_x \cdot \dot{V}/P_x$, that is, the virtual volume of plasma complete cleared of that substance in one minute's time becomes equal to the volume of plasma required to supply the quantity $\underline{x}$ excreted in the urine each minutes's time. The use of the generic definition of renal clearance as the product $E_x \cdot RPF$ would remove much source of confusion among medical students and non-specialists when they have to decifer the meaning of our terminology. In practice, however, the definition of renal clearance became so identified with the equation $C_x = U_x \cdot \dot{V}/P_x$ that most authors, including myself, have used the term "organ clearance" or "total renal extraction" as the generalized expression for renal clearance and continue to reserve the term "renal clearance" to the urinary excretion component.

Clearance techniques permit the measurements of glomerular filtration rate, effective and total renal plasma flow, and the net rates of renal extraction, excretion, reabsorption, secretion and metabolism of endogenous and exogenous substances [1–3, 5, 6]. Earlier workers adopted a rigorous set of experimental rules to validate the use of clearance techniques to measure the above parameters of renal function. These rules have been discussed and justified in the original literature on renal clearance, as well as in more recent reviews on the subject [2, 3]. Unfortunately, as will be discussed below, some of the princi-

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ples and rules governing the use of the technique have been disregarded on some occasions by present day investigators.

The clearance (C) of inulin (In), or in some species, also the clearance of creatinine, provides a fairly accurate estimate of the glomerular filtration rate. The clearance of paraaminohippurate (PAH) provides an estimate of the renal plasma flow (RPF), the so–called effective renal plasma flow (ERPF) which is assumed to measure cortical plasma flow. Accurate determination of total RPF demands the concomitant measure of the renal extraction of PAH. Even estimates of cortical plasma flow by C_{PAH} are subject to gross errors because of the large variations in the extraction rates of PAH by the proximal tubule, particularly during certain experimental maneuvers and in disease states [1–3, 6].

The use of the clearances of inulin (or creatinine) and PAH to determine GFR and ERPF has been recently reviewed in detail [2, 3]. I will address only two additional issues which, in my view, deserve to be re-emphasized: 1) the absolute requirement for near steady–state conditions during clearance periods; and 2) the importance in taking into account the errors in the determination of the value of GFR when interpreting the influence of changes in this parameter on the renal excretion of fluid and electrolytes.

The absolute requirement for near steady–state conditions during the measurements of C_{In} or C_{PAH} imply that during a particular clearance period there is: a) near stability of the plasma levels of infused In and/or PAH, and b) near constancy of the actual values of GFR, RPF and urine flow rates. The reasons for the above requirements are quite obvious and can be briefly summarized as follows.

Stability of plasma levels. If plasma levels of inulin or PAH are rapidly falling or rising during a clearance period, the value of P_{In} (or P_{PAH}), determined by a single sampling of blood, will not necessarily reflect the value of P_{In} (or P_{PAH}) during the course of the clearance period. Therefore, the value of C_{In} ($U_{In} \cdot \dot{V}/P_{In}$) may not represent the true value for GFR in that period. Large variations in P_{In} (or P_{PAH}) may result from inadequate priming or constant infusion of inulin as well as from rapid fluctuations of GFR, RPF, or extraction of PAH. In many present day articles, a reader is at loss when he/she attempts to verify whether plasma levels of inulin or PAH were stable during the course of a published experiment, since plasma levels of these markers are usually not reported, and in many instances the authors do not make a statement regarding this issue. Small unavoidable fluctuations in P_{In} (or P_{PAH}) may be taken into account by sampling blood in the middle of the clearance period, but even then they may contribute to the intrinsic errors in the measurement of GFR and RPF (see below).

Consistency of actual values. The requirement for a stable GFR and/or RPF during the course of a clearance period is due to the fact that measurements of C_{In} (or C_{PAH}) are not cumulative but represent an average during the clearance period. Therefore, if an experimental maneuver has, for example, a transient effect which raises GFR (or C_{PAH}), and if this effect is shorter than the duration of the clearance period, then the values of C_{In} (or C_{PAH}) will clearly underestimate or may even completely miss the increase in GFR (or C_{PAH}). Cases in point in this regard are some recently published clearance studies with atrial natriuretic factor (ANF), a novel endogenous peptide(s) which markedly increases sodium excretion as much as 40-fold and GFR by 30 to 40% in intact mammals [8]. Bolus i.v. administration of this peptide to rats leads to increases in sodium excretion which are of rapid onset, reach a peak about 5 min after administration, and subside towards control levels 10 to 20 min after injection [9]. In a 20 min clearance period, the increase in Na excretion will be readily apparent even if the increase lasted only 5 min, since this is a cumulative measurement. On the other hand, if the increase in GFR also lasted for only 5 min, then it may be missed completely by the measure of C_{In} in the 20 min clearance period. Such experimental errors in the measurement of GFR may have important consequences. In the example above, it led to the postulate that ANF must have a direct tubular effect since the natriuresis apparently occurred without a detectable, statistically significant change in C_{In} [9]. In reality, steady–state measurement of the renal effects of ANF clearly show that this peptide markedly increases GFR and, therefore, this action contributes to the ANF-induced natriuresis [8, 10–12]. Steady–state filtration rates during the clearance period are a sine-qua-non to validate the use of the clearance of inulin (or creatinine) as an estimate of GFR. The same principle should be applied when PAH is used to estimate the value of RPF.

Rapid change of urinary flow rates. Measured values of C_{In} or C_{PAH} may also markedly overestimate or underestimate the true values for GFR and ERPF when urine flow rates change markedly during the course of the clearance period. Again, recent studies with ANF illustrate some of the pitfalls which may be incurred under conditions of rapidly changing urine flow rates. If in the example given above, the clearance period lasted only 5 to 10 min and was initiated concomitantly or immediately after the bolus administration of ANF, then, with rapidly increasing urine flow rates, C_{In} will markedly overestimate the increase in GFR. Indeed, inulin previously concentrated in the tubular lumen and urinary tract in the antidiuretic control conditions will be washed–out by the ANF-induced increase in tubular and urinary flow rates and, in this manner, artificially raise U_{In} and C_{In}. Using such experimental conditions, it has been reported that ANF may increase GFR by as much as threefold [13]. In fact, studies in steady–state conditions demonstrate that the increase, albeit highly significant, is at most 30 to 40% [8, 11, 12]. The same experimental pitfall may be incurred when C_{PAH} is measured during rapidly changing urine flow rates.

Even in ideal conditions, the possible error in the measurement of GFR or RPF may be as high as 10% [2, 3]. This error is particularly important when one considers relationships between filtered loads and urinary excretion rates of fluid and electrolytes. For example, a 10% measurement error in a GFR of 100 ml/min represents 10 ml/min. In a particular experimental maneuver in which urine flow triples from 1 to 3 ml/min and no variation in C_{In} is experimentally detected, the increase in urine flow may still be a small fraction (20% in the present example) of what could possibly have been an unmeasureable increase in GFR. Consequently, a non-detected increase in GFR could have contributed to the increase in urine flow rate in the theoretical example given above. Except in conditions in which sodium excretion is a large fraction of its filtered load, a natriuretic effect without a detectable increase in GFR does not necessarily imply a direct inhibition of tubular transport. Dem-

onstration of the latter must be obtained by more direct techniques such as micropuncture, microperfusion or isolated tubule perfusion.

In clearance studies in which relationships between filtered loads and excretion rates are considered, the meaning of glomerulo-tubular balance must be clearly understood [14–16]. Glomerulo-tubular balance may be erroneously construed as indicating that changes in GFR do not lead to changes in excretion rates because of the proportional increase in tubular reabsorption. Even if perfect glomerulo-tubular balance is attained—a perfection that only biologists, not nature, strive for—changes in GFR per se will always lead to changes in excretion rates. Indeed, if for example, the filtered load of sodium doubles and tubules adapt perfectly by doubling the reabsorption rate of this ion, urinary sodium excretion will still be twice as great as before the increase in GFR. Theoretically, perfect glomerular balance is defined as an increase in reabsorption rate exactly proportional to the increase in filtered load. The concept implies that only fractional, not absolute, excretions rates remain constant and, as pointed out above, even this is an idealized proposition. Glomerulo-tubular balance may be altered in one or another direction by direct changes in tubular transport function, or by hemodynamic means which indirectly decrease the capacity of certain tubular segments to adapt to changes in load [14–16]. For example, it has been recently shown that, whereas a wash-out of the inner medulla does not lead to an increase in sodium excretion per se, it has a major magnifying effect on the natriuresis resulting from an increase in load to the distal nephron [17]. This phenomenon is due to a decreased capacity of the papillary thin loops of Henle to fully adapt to increases in load when the hypertonicity of the inner medulla is decreased [17]. Therefore, in clearance experiments, an increase in fractional sodium excretion should not be construed as necessarily indicating a direct sodium transport inhibitory effect of a particular substance or experimental maneuver.

In addition to classical renal clearance methods, several derived techniques were introduced to indirectly localize and/or assess transport function of particular nephron segments in whole organ experiments. Included among these techniques are: stop-flow, to localize transport functions in "proximal" and "distal" nephron segments [18]; precession clearance, a technique which is useful to study peritubular uptake of administered substances in vivo [19]; lithium clearance, to estimate "proximal" reabsorption of sodium [20]; measurements of free water clearance, related to proximal delivery, to assess effects of diuretics on Henle's loop [3, 21]. Description and analysis of the above methods are beyond the scope of the present review [3, 22, 23]. The present day usefulness of these methods resides mostly in assessing segmental nephron function in experimental conditions in which more direct technologies cannot be used. In particular, some of the above clearance methods are applicable to humans and in this sense are of unique importance. Nevertheless, without exception, whole organ measurements to assess segmental nephron function have serious drawbacks and, in general, can provide only rough semi-quantitative estimates of the function of defined nephron segments.

Clearance methods are not only useful for intact animal experiments, but are an important experimental tool in studies of isolated perfused kidneys. Use of the organ in isolation permits the study of renal function under conditions in which

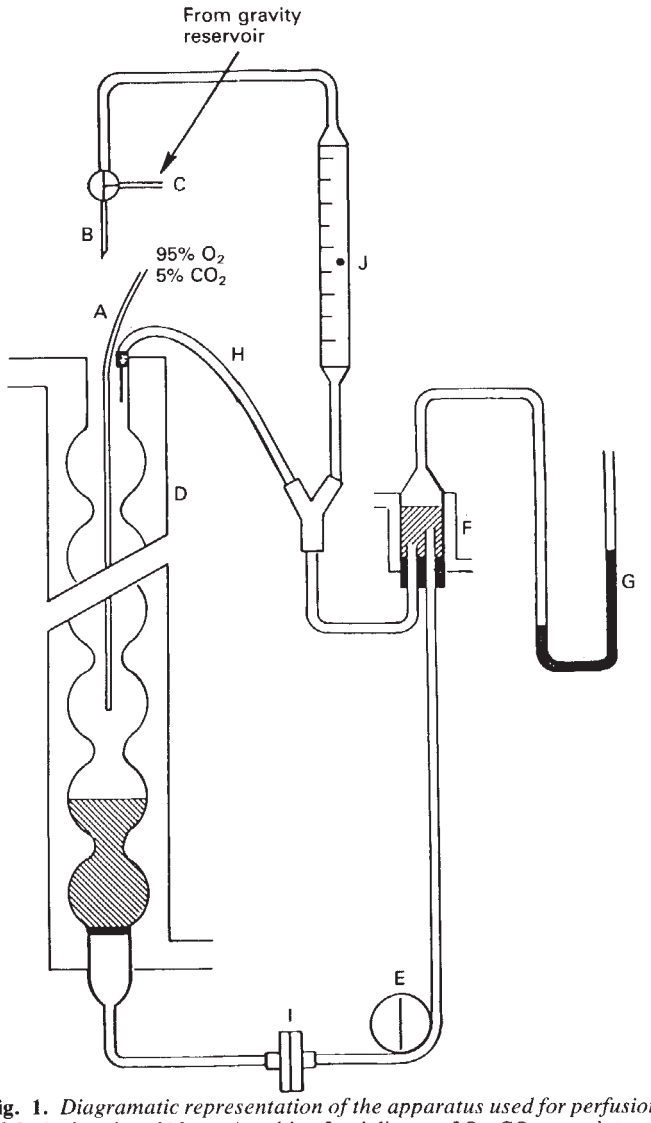

Fig. 1. *Diagramatic representation of the apparatus used for perfusion of the isolated rat kidney.* A, tubing for delivery of O_2–CO_2 gas mixture. B, arterial cannula (18 gauge blunted metal needle). C, connection to gravity reservoir placed about 85 cm above the chamber D (Not shown). D, perfusion–oxygenation chamber: double-walled 60 cm glass condenser. E, non-pulsatile perfusion roller-pump. F, double-walled glass bubble-trap chamber; G, mercury manometer which may be substituted by a pressure transducer if continuous recording of pressure is desirable. H, side-arm resistance (22 gauge needle). I, filter holder containing a 14 μm millipore filter. J. Brooks flowmeter which may be substituted by an electromagnetic flowmeter if continuous recording of renal perfusate flow is desirable. Connecting tubes are made of tygon. The composition of the perfusate is given in Table 1. For details see references 25, 27–30.

variables can be changed in a controlled manner. Furthermore, systemic influences are eliminated while the anatomical, biochemical and at least some of the functional characteristics of the kidney are preserved. In the last part of this review, I will consider the major characteristics of the isolated perfused rat kidney preparation and analyze some of the advantages and disadvantages of its use in studies of renal function and metabolism. In this regard the reader is also directed to two recent reviews on the subject [24, 25].

Table 1. Composition of the perfusate.

Ions	Concentration
Na^+	141 mM
K^+	5.4 mM
Ca^{++}	2.0 mM
Mg^{++}	2.4 mM
Cl^-	120 mM
HCO_3^-	25 mM
Inorganic phosphate	1.5 mM
SO_4^-	2.4 mM
Organic substances	
Glucose	1.0 mg/ml
AA Mixture	
L-methionine	0.5 mM
L-alanine	2.0 mM
Glycine	2.0 mM
L-senine	2.0 mM
L-proline	2.0 mM
L-isoleucine	1.0 mM
L-aspartic acid	3.0 mM
Creatinine	0.5 mg/ml
Albumin	7.5 g/dl
pH	7.4

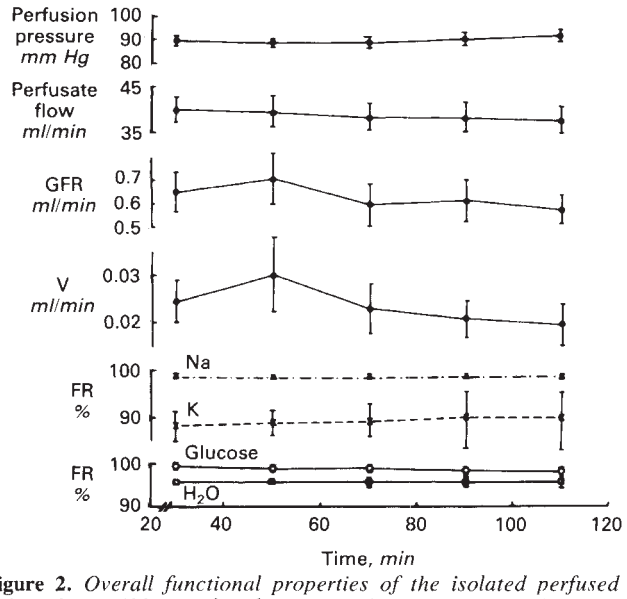

Figure 2. *Overall functional properties of the isolated perfused rat kidney during 120 min of perfusion.* Results are mean ± SE ($N = 6$). The average kidney weight was 1.2 g. V, urine flow rate; FR, fractional reabsorption. Reproduced by permission [25].

Isolated perfused rat kidney

Many physiological and biochemical aspects of renal function have been studied in the past decade using the isolated rat kidney perfused with an artificial cell-free medium. The technique in itself has been available since the beginning of the century. In more recent times, the preparation used by investigators in the field is essentially derived from that initially developed by Weiss, Passow, and Rothstein in the late 50s [26]. These authors used the technique to demonstrate for the first time that red cells are not essential for autoregulation of flow in the kidney. Isolated kidneys were used sparingly in the 60s mainly because of their poor functional performance and lack of stability. In the early 70s, however, two groups of investigators introduced modifications which markedly improved the stability and at least some of the functional characteristics of the preparation [24–31]. In addition to improvements in surgical techniques, the key modifications were: 1) use of high perfusate albumin concentrations (7.5 g/dl) to increase the oncotic pressure of the perfusate and in this manner improve proximal reabsorption of fluid and electrolytes [25, 28–30]; 2) addition of a mixture of amino acids to the perfusate in order to improve the stability of the preparation [25, 29, 30]; and 3) introduction of an in-line filter in the perfusion apparatus to remove debris originating from the kidney which tended to markedly decrease the GFR during the course of perfusion [24, 31]. Figure 1 depicts a diagramatic representation of the perfusion system developed in my laboratory, while Table 1 presents the composition of the perfusate used in our studies with the isolated kidney.

Figure 2 illustrates some of the main overall functional characteristics of our preparation when it is perfused at a constant effective perfusion pressure (pressure at the tip of the arterial cannula) of about 90 mm Hg. As can be seen, during two hr of perfusion the functional parameters depicted in the figure remain relatively stable. I will consider separately the hemodynamic and tubular functions of the preparation.

Hemodynamic characteristics of the isolated perfused rat kidney

The renal perfusate flow rate (RPF) of the isolated preparation is very high (35 to 45 ml/min) mainly because of the low viscosity of the perfusate as compared to blood with a normal hematocrit [25]. In addition, it cannot be ruled out that because of lack of innervation and/or circulating vasoconstrictor substances, renal vasodilation may contribute to the increase in RPF. Nevertheless, the isolated kidney is able to maintain at least some vascular tone, since classical vasodilator substances such as acetylcholine and bradykinin are able to further increase RPF and decrease renal vascular resistance in the preparation. The high RPF is essential to maintain proper oxygenation of the renal tissue since the perfusate carries only dissolved oxygen. It has been demonstrated that a RPF of less than 10 to 20 ml/min markedly compromises the oxygenation of isolated perfused rat kidney preparations [32]. Even with the high flow of 40 ml/min, some structures of the kidney may be in hypoxic conditions.

The very high perfusate flow, together with a somewhat diminished GFR, results in an exceedingly low filtration fraction (0.01 to 0.03) [24, 25]. Consequently, contrary to the intact kidney, peritubular oncotic pressure fails to increase significantly in the isolated kidney. This was the major cause of the high rates of urinary sodium excretion, the high intraluminal pressure and low reabsorption of fluid and electrolytes in proximal tubules in early preparations in which the perfusate had an oncotic pressure similar to that of plasma. Thus, we increased the oncotic pressure of the perfusate to levels similar to those obtained in peritubular blood of intact rats (about 40 to 50 mm Hg). This was accomplished by raising the perfusate albumin concentration to 7.5 g/dl [25, 28–30]. In this manner we obtained a prompt decrease in absolute and fractional sodium excretion and a normalization of intraluminal pressure and fluid

Table 2. Function of cortical nephron segments in the isolated perfused rat kidney.

Function	Nephron segment			
	End proximal	Earliest distal	Latest distal	Ureteral urine
Transit time sec	17.7 ± 0.6 (19K)	37.2 ± 1.0 (10K)	82.3 ± 3.0 (8K)	—
Intratubular pressure mm Hg	14.3 ± 1.2 (7K)	14.9 ± 0.9 (6K)	—	—
SNGFR nl/min	27.2 ± 2.2 (13K)	25.2 ± 1.9 (5K)	—	—
^{3}H-PEG (TF/P)	2.78 ± 0.16 (32)	3.86 ± 0.57* (9)	5.97 ± 0.59** (10)	19.0 ± 3.1** (15K)
Na (TF/P)	0.98 ± 0.02 (14)	0.88 ± 0.06* (8)	0.65 ± 0.10* (6)	0.48 ± 0.05 (10K)
Na/^{3}H-PEG (TF/P)	0.34 ± 0.01 (14)	0.26 ± 0.04* (8)	0.12 ± 0.03** (6)	0.03 ± 0.01** (10K)

Values are mean ± SE. Abbreviations are: (#K) the number of kidneys and values given are an average of at least three determinations on each kidney; (#) the number of micropuncture samples obtained in at least five kidneys; ^{3}H-PEG, tritiated polyethylene glycol, a marker of glomerular filtration and fluid reabsorption; SNGFR, single nephron glomular filtration rate; (TF/P) tubular fluid to perfusate concentration ratio; * $P < 0.05$ and ** $P < 0.01$ in relation to value of the preceding nephron segment. Modified from [29].

and electrolyte reabsorption in the proximal convoluted tubule [25, 28–30], (Table 2).

The high RPF is also partly responsible for the inability of the isolated kidney to properly concentrate the urine even in presence of ADH. Usually, in the absence of ADH the urine is isotonic or slightly hypotonic. This inability to properly dilute the urine is most likely due to a defect in function of the thick ascending limb of Henle's loop. In the presence of ADH, the urine is also nearly isotonic and only in very special conditions it may become slightly hypertonic. Obviously, the high RPF will lead to a wash–out of the inner medulla and in this manner inpede the formation of a concentrated urine [25].

Figure 2 also shows that the GFR of our preparation is lower than normal, a phenomenon which may result in part from the high oncotic pressure of the perfusate. Indeed, by raising the effective hydrostatic perfusion pressure to 120 mm Hg or perfusing the kidney with 5 g/dl of albumin, the GFR tends to normalize [25, 28, 33]. In these conditions, however, the preparation becomes markedly natriuretic and, as importantly, its stability is compromised [25, 28, 33]. Therefore, in the present stage of development of the isolated kidney, the investigator must compromise between an unstable and natriuretic preparation with a near normal GFR, or a stable preparation with near normal sodium excretion but lower than normal GFR. For the purposes of most of our studies we chose the latter alternative. It should be pointed out that, although the increased oncotic pressure is likely to be an important factor in the decreased GFR of the preparation, it cannot be ruled out than an abnormal tonus of the afferent and/or efferent arterioles may alter glomerular dynamics. In this respect it is of interest that atrial natriuretic factor, which increases efferent arteriolar resistance, markedly increases the GFR of the preparation [10, 34] (Fig. 3A). Low concentrations of angiotensin II, another preferential efferent arteriolar constrictor, also increases the GFR of the preparation, albeit to a lesser degree than atrial natriuretic factor [35]. Therefore, it is possible that a lower than normal efferent arteriolar tonus (perhaps due to a lack of angiotensin II) contributes to the low GFR of the preparation.

In view of the rather marked abnormalities described above, the question of the usefulness of the preparation to study renal hemodynamic and vascular functions in the isolated kidney does not have a simple answer. Obviously, the preparation is inadequate to quantitate renal hemodynamic parameters per se. However, the isolated kidney may provide very useful qualitative or semi-quantitative information regarding some renal hemodynamic and vascular properties. In some cases, judicious use of the preparation allows insights which cannot be obtained in intact animal experiments or in other in vitro preparations. A pertinent example is the already mentioned classical demonstration by Weiss, Passow and Rothstein that the isolated kidney is able to autoregulate flow when perfused with a cell–free medium [26]. Thus, the early postulate that red cells are essential for autoregulation (plasma skimming theory) was tested and found erroneous by this simple and decisive experiment.

The simplicity of the preparation and the yield of information obtained makes the isolated kidney a particularly useful preparation to gain at least initial knowledge on sites and overall mechanisms of action of renal vasoactive substances. Two pertinent examples in this regard are the studies of Davalos et al which discovered that angiotensin II is a preferential efferent arteriolar vasoconstrictor [35], and the studies of my laboratory, which unveiled the vasoactive properties of atrial natriuretic factor (ANF), as well as the relationships between the renal hemodynamic and natriuretic effects of this hormone [8, 10, 34]. Figure 3 illustrates some of the results obtained with ANF in the isolated perfused rat kidney. Panel A shows that atrial extract increases renal vascular resistance, GFR, filtration fraction, diuresis, natriuresis and kaliuresis in the preparation. These effects are abolished when the kidneys are perfused with a very low calcium concentration. These results led to the conclusion that ANF is a renal vasoactive substance with a preferential effect on the efferent arteriole [10]. The results also indicated that the ANF-induced natriuresis is due at least in part to an increase in sodium load into a washed–out inner medulla [10]. Similar results were obtained with a synthetic form of ANF [34]. Panel B shows that, whereas ANF increases slightly but significantly the renal vascular resistance of the vasodilated isolated kidney, it stongly counteracts the effects of other vasoconstrictors in the preparation. This led to the conclusion that ANF behaves as a functional partial agonist, that is, a weak agonist and a powerful antagonist of renal vasoconstrictors [10].

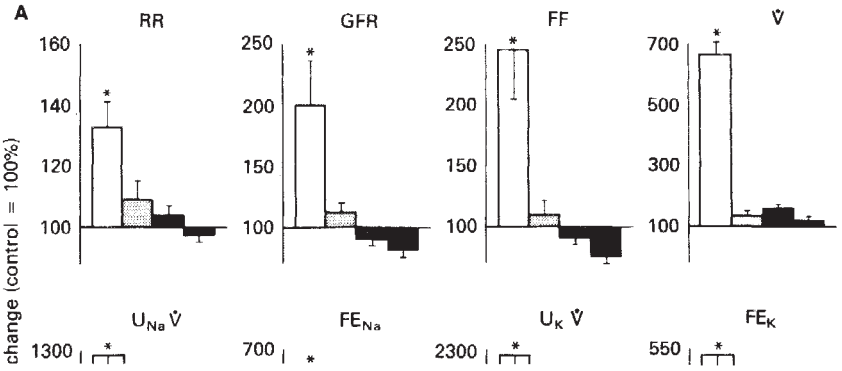

Fig. 3A. *Effects of atrial natriuretic factor (ANF), on renal function and renal vascular resistance in the isolated perfused rat kidney.* Relative changes in renal function parameters after atrial extract (AE) or ventricular extract (VE) in isolated rat kidneys perfused in presence of normal [Ca] (2mM) or very low

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L-senine	2.0 mM
L-proline	2.0 mM
L-isoleucine	1.0 mM
L-aspartic acid	3.0 mM
Creatinine	0.5 mg/ml
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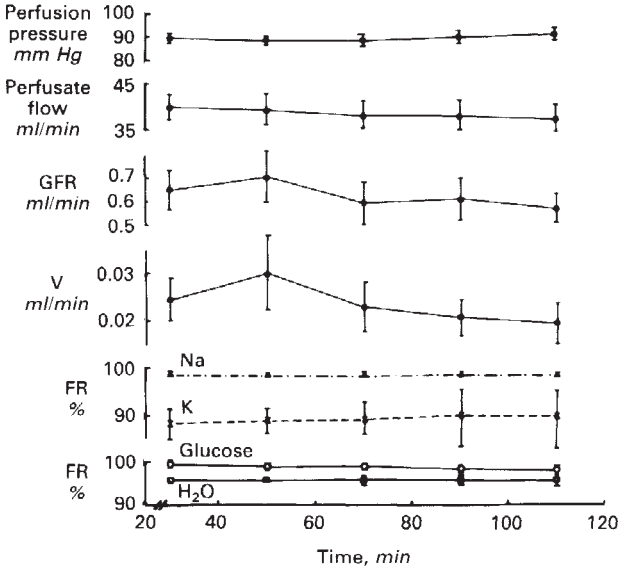

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The very high perfusate flow, together with a somewhat

high net reabsorption of potassium under normal conditions, but Silva et al have shown that the isolated preparation is able to net secrete potassium when donor rats are kept on a high K diet [37]. Finally, glucose reabsorption is essentially complete, indicating the usefulness of the preparation in studying reabsorption and uptake of organic solutes. Indeed, the reabsorption of amino acids and uptake of proteins are within normal limits in the preparation [7, 25, 38]. The isolated kidney is responsive to proximal, loop and distal diuretics [24, 39], as well as to atrial natriuretic factor which, as mentioned above, induces natriuresis at least in part by renal hemodynamic means [10, 34].

We made an extensive micropuncture study of the isolated rat kidney to characterize the functions of superficial cortical nephrons of the preparation [29]. The technique of preparing the isolated kidney for micropuncture studies is described in detail [29]. Table 2 summarizes some of the findings. Single nephron (SN) GFR in superficial nephrons is only slightly lower than in intact Sprague–Dawley rats. In view of the lower total GFR, deeper nephrons SNGFR must, by unknown reasons, be markedly reduced [29]. Proximal intratubular pressure is within normal limits but distal tubular pressure is markedly elevated in comparison to normal, a phenomenon which is probably due to a decrease in the reabsorption of fluid in Henle's loop.

As shown in Table 2, proximal reabsorption of fluid and sodium is within normal limits. In addition, in this nephron segment, chloride and potassium concentration profiles as well as transepithelial potential differences and specific resistances are within normal ranges [25, 29]. From these data, it is clear that the functions of superficial proximal convoluted tubules are well preserved in the isolated perfused rat kidney preparation.

The major tubular functional abnormalities of the preparation are found in the superficial loop of Henle, defined here as the segment between the end proximal convoluted tubule and the early distal tubule. As shown in Table 2, fluid reabsorption is well below normal ranges and the loop is unable to decrease Na concentration to the low levels found in vivo. Potassium handling by the loop is also abnormal since K concentrations in early distal tubule are elevated [29]. It is of great interest that Alcorn et al [40], Brezis et al [41], and Schurek and Kriz [42] found that the major morphological abnormality of the isolated kidney resides in the thick ascending limb of Henle's loop, in which clear signs of anoxia and cell degeneration are present. The reasons for the functional and morphological lesion in the thick ascending limb of Henle's loop are not entirely clear, but these structures are particularly susceptible to anoxic conditions in view of their distance from nourishing bundles of vasa recta vessels [41, 42]. This condition may be exacerbated in vitro conditions because the oxygen delivery from vasa recta is deficient and/or the access of oxygen to the thick ascending limbs of Henle's loop is further hindered by interstitial swelling [40–42]. In this regard it has been reported that addition of washed red cells to the perfusate markedly improves the morphological abnormalities in the thick ascending limb of Henle's loop [42]. Unfortunately, this improvement is not accompanied by a marked increase in the sodium–reabsorptive capacity of the isolated preparation [42]. Despite the abnormalities, active transport of NaCl in the thick ascending limb does not cease completely since loop diuretics (such as furosemide) are still able to elicit a major natriuretic response in the preparation [24, 39]. It is likely, however, that the poor functional performance of this segment is responsible for the inability of the kidney to properly dilute the urine [25].

It has been observed that there is a depletion of glutathione levels during the course of perfusion, a phenomenon which may contribute to the functional and morphological abnormalities of the loops of Henle in the isolated kidney [40, 41]. It has also been reported that inclusion in the perfusate of amino acids, which are precursors of glutathione synthesis, stabilize kidney function for up to four hrs of perfusion [43]. However, inspection of the data reveals that, under this condition, the functional properties of the preparation are not entirely adequate, albeit better than in kidneys perfused entirely without amino acids [43]. Addition of the amino acid precursors of glutathione synthesis to the amino acid mixture shown in Table 1 does not further improve the stability of our preparation (M.J.F. Camargo and T. Maack, unpublished results). More work is needed to determine the role of the depletion of glutathione stores in the functional abnormalities of the isolated perfused rat kidney.

The distal tubule is able to compensate at least in part for the deficient loop reabsorption. As shown in Table 2, superficial distal tubules are able to reabsorb 36% and 54% of the load of fluid and sodium, respectively. The distal tubule is also able to significantly decrease sodium and chloride concentrations to values significantly below those found in the early distal tubule and is able to secrete potassium [29].

Collecting tubule function was not assessed, but, undoubtedly, these tubules are able to further reabsorb fluid and to maintain steep lumen–interstitial Na gradients (Table 2) [29]. In micropuncture studies the final urinary excretion of sodium is usually higher than in the preparation depicted in Figure 2. The probable reason for this discrepancy is the greater manipulation of the kidney when it is prepared for micropuncture studies as compared to kidneys in which only overall functions are determined [25, 29].

From the foregoing, it is clear that the preparation is suitable to study functional properties of proximal convoluted tubules. Thus, transport of organic substances such as glucose, amino acids, proteins and peptides have been successfully studied in the isolated preparation [7, 24, 25, 27, 30, 38]. Studies of functions which reside in the distal nephron are more problematic because of the described deficiencies of Henle's loop function. This does not completely preclude the use of the preparation for this purpose, but the results cannot be quantitatively extrapolated to intact animals. In the last instance, judgement as to when to use the preparation must be determined by the type of information that is being pursued. In general, I have adopted the criteria of using the isolated perfused kidney preparation to gain information on pathways and mechanisms of renal function which could not be obtained, at least initially, in intact animal experiments.

Renal metabolism and hormone-receptor interactions

One of the major applications of the isolated perfused rat kidney preparation is in studies of renal metabolic functions. The preparation has major advantages over other in vitro procedures (such as, kidney slices) in the sense that anatomical and functional properties are preserved and variables can be changed in a controlled manner. Studies in the isolated kidney

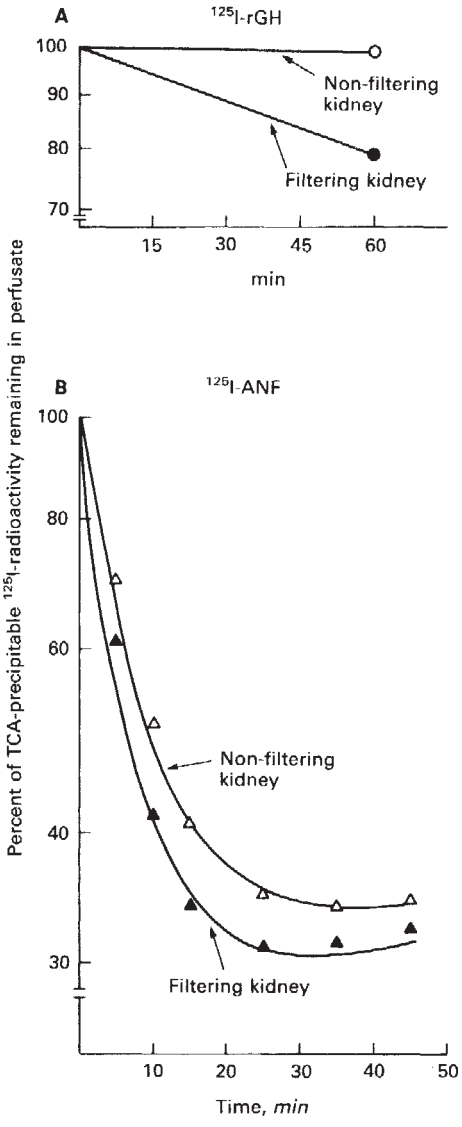

Fig. 4. *Renal uptake of rat growth hormone (rGH) and rat atrial natriuretic factor (ANF) in the isolated perfused rat kidney.* **A.** Perfusate decay of ^{125}I-rGH in filtering and non-filtering isolated kidneys. The decay of ^{125}-TCA precipitable radioactivity is due to complete hydrolysis of ^{125}I-rGH to amino acids via filtration, luminal uptake and lysosomal catabolism of the hormone [30]. The hydrolysis of ^{125}I-rGH in non-filtering kidneys is negligible. Data from [30]. **B.** Perfusate decay of ^{125}I-ANF in filtering and non-filtering kidneys. Contrary to the observations with rGH, the perfusate decay of ANF in non-filtering kidneys is only slightly smaller than in filtering kidneys. This indicates that the bulk of the renal uptake of ANF does not occur by the filtration process. The perfusate decay of ANF is also much faster than that of rGH and is due to the binding of ANF to its renal receptors [50].

on renal metabolic pathways, substrate utilization as well as studies on correlations between renal metabolism and transport provided important new insights in these fields [24, 44–46]. Modern spectrometric and nuclear magnetic resonance techniques are applicable to the isolated kidney [47, 48]. Therefore, one can expect a significant future growth in the uses of the preparation to study renal biochemistry.

We have used extensively the isolated perfused rat kidney to study pathways, kinetics and mechanisms of renal uptake, transport and metabolism of proteins and peptides [7, 38, 49]. For this purpose, in addition to the normal filtering isolated perfused rat kidney, we developed a non-filtering isolated kidney model [25, 30] to differentiate between phenomena which occur primarily via the filtration route (luminal binding, uptake, intracellular transport and metabolism) from those which take place at peritubular sites or in glomerular and vascular tissue. In this model, filtration is abolished by simply raising the oncotic pressure of the perfusate to levels sufficient to fully counteract the hydrostatic filtration pressure. In practice this is accomplished by increasing the albumin concentration of the perfusate to 10 g/dl and slightly reducing the effective perfusion pressure to 70 to 80 mm Hg [25, 30]. The advantage of this preparation over other non-filtering kidney models is that filtration is effectively abolished, perfusion flow rate is largely unaffected and the metabolic integrity of the cells is mostly preserved [25, 30]. As in other non-filtering preparations, the disadvantage is that the procedure does not permit localization of the phenomena within the heterogenous structure of the kidney. Nevertheless, if such localization is obtained by independent means, such as, autoradiography or histochemistry, then even this disadvantage can be overcome.

Figure 4 gives an example of the pathways (luminal vs. extraluminal) of the renal processing of two hormones—rat growth hormone (rGH) and rat atrial natriuretic factor (ANF)—obtained in filtering and non-filtering isolated perfused rat kidneys. Panel A shows that the perfusate decay of rGH occurs almost exclusively by the luminal route since uptake and hydrolysis of the hormone in the non-filtering kidney is practically negligible [30]. Further studies using the isolated kidney demonstrated that rGH is hydrolyzed in proximal tubular cells by an endocytic process, which ultimately delivers the absorbed hormone to lysosomes [30]. In these organelles, absorbed proteins, including rGH, are hydrolyzed to completion and the resulting amino acids are then released back to circulation [7, 30, 38, 49]. Some hormones which have receptors at the contraluminal membrane (such as insulin and parathyroid hormone) are also partially hydrolyzed at peritubular sites [7, 38]. Panel B shows that, in contrast to rGH, the bulk of the renal uptake of ANF does not occur via the filtration–luminal uptake route. Indeed, the rate of perfusate decay of ANF in the non–filtering kidney is only slightly lower than in the filtering kidney [50]. It is also apparent from Figure 4 that the rate of renal uptake of ANF is far greater than that of rGH. As of this writing, the exact location of the site(s) of renal uptake of ANF has not been determined, but further studies clearly indicated that the uptake of ANF by the isolated kidney is due to the binding of the hormone to specific renal receptors [50].

The isolated perfused rat kidney has also been successfully applied to characterize the nature of renal metabolites of proteins and peptides as well as in studies of factors which influence the kinetics of renal uptake, transport and metabolism of these organic substances [7, 38, 49]. In principle, the same approaches can be applied to other organic substances. Furthermore, the initial studies on ANF-receptor binding suggest that the preparation is also a very useful experimental tool with which to study renal receptor physiology.

In summary, isolated perfused rat kidneys are useful experimental preparations with which to study some aspects of renal

function and metabolism. When the isolated kidney is judiciously employed, it can become an important experimental tool in studies of renal physiology and pathophysiology.

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Reprint requests to Thomas Maack, M.D., Department of Physiology, Cornell University Medical College, 1300 York Avenue, New York, New York 10021, U.S.A.

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